Brit. J. vener. Dis. (1976) **52**, 124

SESSION FOUR: *Microbiological and serological research in* Neisseria *infections*

Immune response to a purified cytoplasmic protein of *Neisseria gonorrhoeae*

J. B. G. KWAPINSKI, C. CATES, P. LANDOLFO, A. CHENG, AND A. R. RONALD
From the Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada

Summary

This paper describes studies based on the hypothesis that the immunogenicity of the gonococcus is impaired by a component toxic to immunocytes. Cytoplasm of colony type 1 gonococci was found to contain a protein fraction β^{+t} not present in colony type 4 gonococci. From the results of further analysis it is tentatively deduced that β^{+t} consists of a toxic component $T\beta^{-t}$ and an immunogen.

Introduction

This research was a continuation of our investigations guided by a hypothesis (Kwapinski and Cheng, 1975) that the immunogenicity of the gonococcus is impaired by a highly toxic component which is closely linked with an immunogen and which adversely affects immunocytes. Thus, an artificial separation of the immunogen from the toxic component would uncover its immunizing power. It was further postulated that the source of the desired immunogen would be the inner content of the gonococcus, and that during a natural gonococcal infection the bacterial inner content is released by enzymes of macrophages, but in this event the immunogen remains linked to the powerful toxic component and is ineffective.

Experimental procedures

The inner content, or cytoplasm, was obtained from six cultures of gonococci, including Kellogg's strain F62 and five other cultures received from active cases of gonorrhoea. Before the cytoplasm production, 95 per cent. pure clonal colonial forms 1 and 4 were separated after thirty to sixty selections and subcultures on nutrient-supplemented medium. The cytoplasm was obtained by breaking the gonococci at 20 Kz sec, followed by differential centrifugation, and filtration through 0·45 μ membranes, as described earlier.

To determine the number of antigenic components, the cytoplasms were subjected to an electric current at 10 mA/cm, using a two-dimensional immunoelectrophoresis (Weeke, 1973), 1·2 per cent. agarose (Indubiose) as a gel medium, and antisera produced in albino rabbits against whole cytoplasms.

Repeated testing by this method revealed that the cytoplasm derived from the clonal type 1 contained twelve to fourteen electrophoretically different antigenic constituents, as opposed to nine to eleven antigens existing in the type 4 cytoplasm, and that they differed mainly by two or three flat peaks found in an area extending from a 3 cm. distance from the cathode end over a length of 2 to 3 cm. towards the anode (Figs 1 and 2).

Guided by these observations, we proceeded to isolate constituents that were only found in type 1 cytoplasm, using isoelectric focusing and polyacrylamide gel electrophoresis (Cheng, Kwapinski, and Ronald, 1974; Kwapinski

TABLE *Amino-acid composition of the cytoplasmic proteins of* N. gonorrhoeae

Amino-acid	*Per cent. molar composition of*		
	β (+t) protein	*β (−t) protein*	*Tβ protein*
Lysine	8·8	31·5	9·3
Aspartic acid	9·9	6·0	10·2
Serine	4·7	9·1	6·5
Glutamic acid	11·7	10·6	12·8
Glycine	11·0	36·8	10·7
Alanine	12·7	6·0	12·7
Arginine	4·1	Trace	5·0
Histidine	1·6	0	2·5
Threonine	5·6	0	6·8
Proline	0·8	0	1·0
Valine	8·3	0	8·4
Methionine	1·5	0	2·6
Isoleucine	5·0	0	5·6
Leucine	8·4	0	9·7
Tyrosine	2·5	0	3·6
Phenylalanine	3·2	0	4·6

Presented at the 28th General Assembly of the IUVDT, Malta, April, 1975
Present address and address for reprints: Prof. J. B. G. Kwapinski, D.M., D.Sc., Department of Microbiology, Kirksville College of Osteopathic Medicine, Kirksville, Missouri 63501, USA
Research supported by a grant from Medical Research Council of Canada

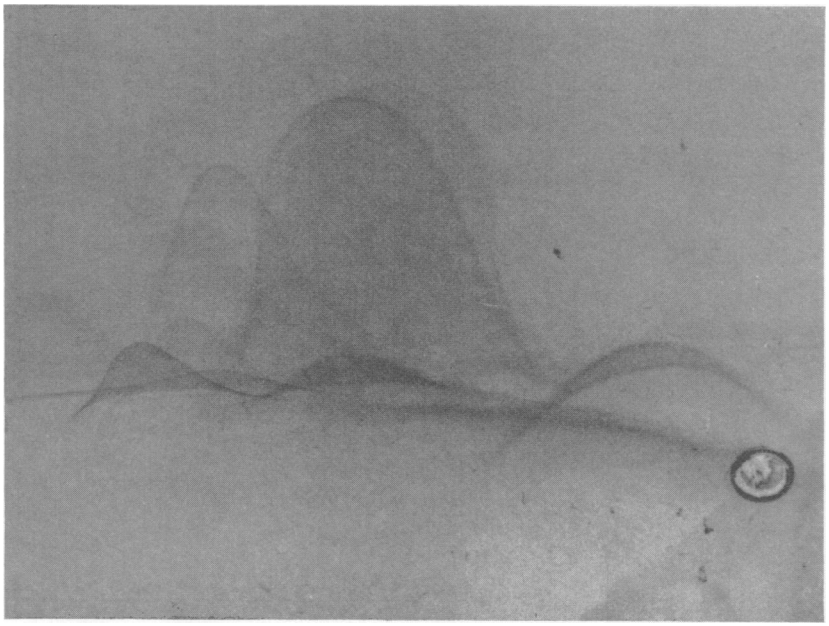

FIG. 1 *Two-dimensional immunoelectrophoresis pattern of Type* 1 *cytoplasm of strain F62*

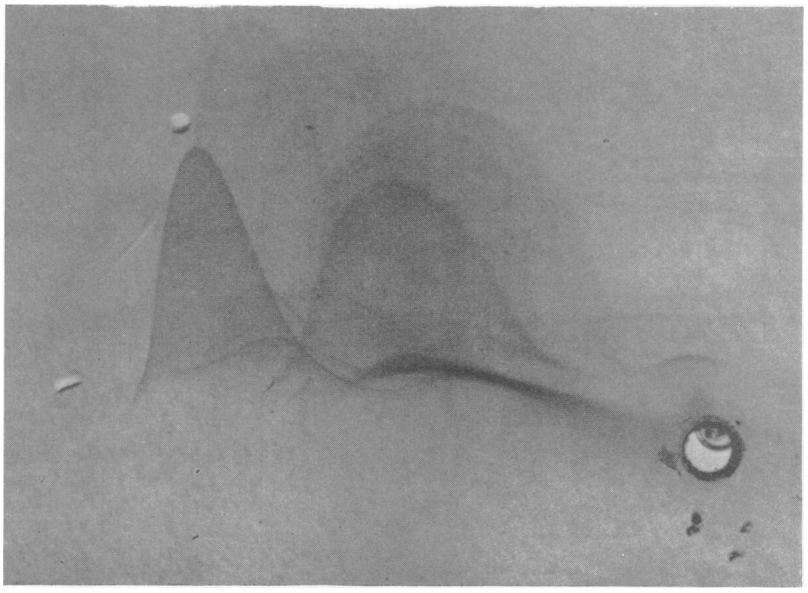

FIG. 2 *Pattern of Type* 4 *cytoplasm of strain F62*

and Cheng, 1975). With the aid of the first procedure, a protein fraction β^{+t} was isolated from type 1 cytoplasm; it proved to be highly toxic for 11-day-old chick embryos. Placed subsequently on a polyacrylamide gel column, the β^{+t} fraction yielded two materials: a non-toxic β^{-t} protein and a toxic Tβ protein. Chemical examinations of all three fractions, obtained from type 1 colonies of the strain F62, by the aid of an Amino-acid Autoanalyser revealed (Table) that the β^{-t} protein consisted of only six or seven types of amino-acids: lysine, aspartic acid, serine, glutamic acid, glycine, and alanine (and traces of threonine), occurring in the molar ratios of 5:1:1·5:1·7:6:1·5. Subjected to sodium dodecyl sulphate (SDS) treatment in an analytical polyacrylamide gel electrophoresis (Weber and Osborne, 1969), this material yielded a single band, and its molecular weight was estimated at 28,000. On immunodiffusion and immunoelectrophoresis carried out with the antisera produced against whole cytoplasm of type 1 strain F62, and against β^{+t} protein, the β^{-t} protein formed a single precipitation band. The β^{+t} protein fraction yielded two different polymer bands on SDS polyacrylamide electrophoresis, with the major component having

an approximate molecular weight of 62,000 to 65,000. On immunodiffusion and immunoelectrophoresis, it formed two bands. Examined in an Amino-acid Analyser this material was found to contain sixteen different amino-acid types including the six amino-acids occurring in β^{-t} (Table). These data coincided well with those obtained from the analysis of amino-acids present in β^{-t} and β^{+t} proteins, recovered from cytoplasms of the strain MB, type 1 of *N. gonorrhoeae*, reported earlier (Cheng and others, 1974).

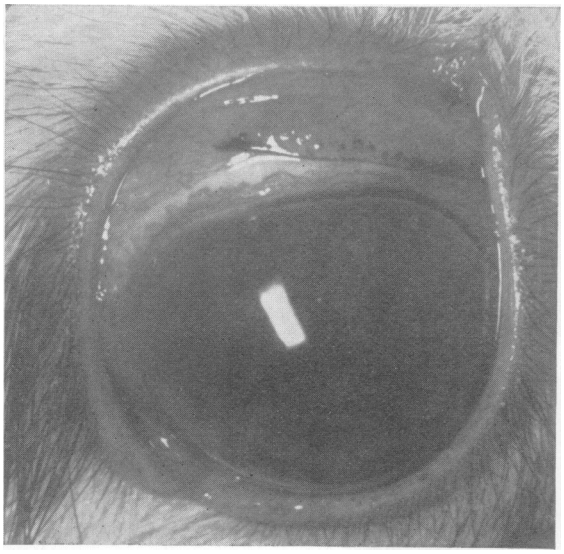

FIG. 3 *Cornea of rabbit pre-injected with β^{-t} and challenged with live gonococci*

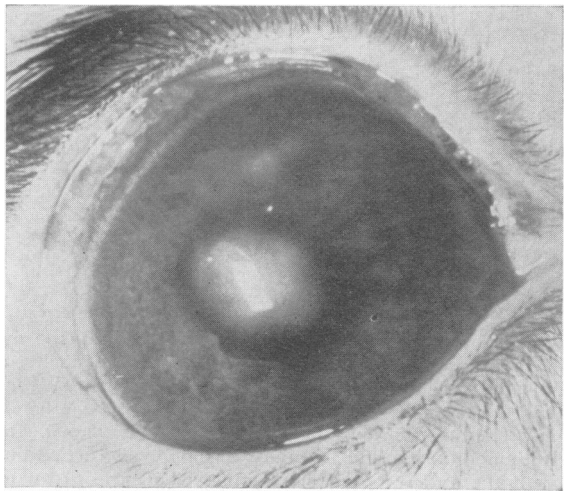

FIG. 4 *Cornea of rabbit pre-injected with formolized gonococci and challenged with live gonococci*

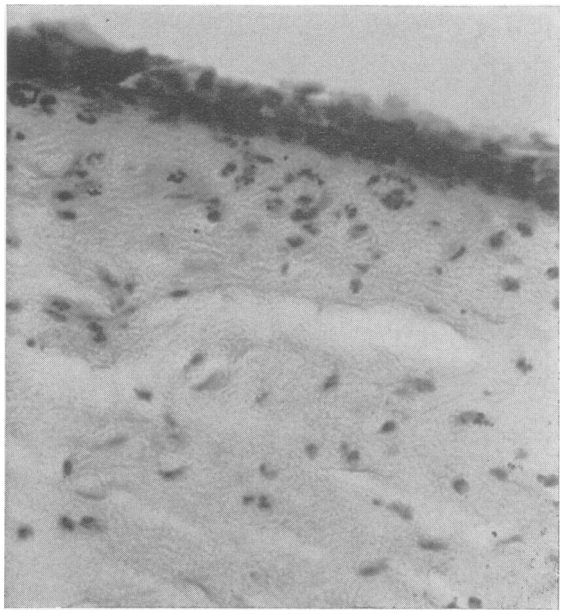

FIG. 5 *Section of cornea from rabbit pre-injected with β^{-t} and challenged with live gonococci*

The toxic protein Tβ consisted of sixteen amino-acids: lysine, aspartic acid, serine, glutamic acid, glycine, alanine, arginine, histidine, threonine, proline, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine, occurring in molecular ratios of 9:10:6:13:11:13:

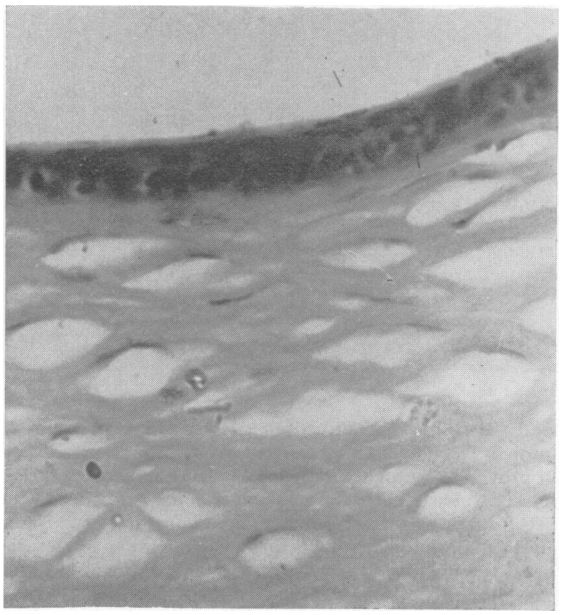

FIG. 6 *Section of cornea from rabbit pre-injected with β^{+t} and challenged with live gonococci*

5:2:7:1:8:3:6:10:4:5. These amino-acids were identical with those found in β^{+t}, at slightly different molar ratios. On immunoelectrophoresis carried out against an anti-β^{+t} antiserum, the Tβ protein produced one distinct band.

We have only indirect evidence that the toxicity of β^{+t} and Tβ proteins depends on peptide(s) composed of valine, leucine, phenylalanine, tyrosine, methionine, and proline. All these amino-acids are missing from the non-toxic β^{-t} protein. However, the presence in both Tβ and β^{+t} of lysine, aspartic acid, serine, glutamic acid, glycine, and alanine, the amino-acids that form β^{-t} protein, suggests that only a part of the latter macromolecule has been detached from the β^{+t} fraction.

Earlier immunological studies have shown that β^{-t} protein, but not β^{+t}, elicited delayed hypersensitivity in guinea-pigs whereas β^{+t} induced greater antibody production in rabbits. For investigations on local immunity, the cytoplasm of formolized gonococci were first injected into the cornea, using alternate eyes of each rabbit for different antigen. In 18 to 21 days thereafter, 10^4 to 10^6 live gonococci were injected into the corneae.

It was observed that within 1 to 2 days cloudy patches appeared on the corneae; but the corneae of rabbits pre-injected with β^{-t} protein cleared completely within 3 to 4 days (Fig. 3). In contrast, the corneae of rabbits pre-injected with other materials cleared partially leaving a central area filled with a dense material (Fig. 4), which proved to contain numerous live gonococci.

Histological examinations of ultrathin sections were made from the corneae taken on the 2nd to 3rd day after the challenge with live gonococci. It was observed in the corneae from rabbits pre-injected with β^{-t} protein that numerous mononuclear cells and some polynuclear leucocytes migrated towards the challenge area (Fig. 5). In contrast, hardly any cellular response was detected in the corneae pre-injected with other antigens (Fig. 6).

Pending further series of similar experiments, it is tentatively concluded that the β^{-t} protein appears to be the sought for immunogen, detached from a toxic component, and capable of eliciting an immune protection against gonococcal infections in rabbits.

References

CHENG, A., KWAPINSKI, J. B. G., and RONALD, A. R. (1974) *Canad. J. Microbiol.*, **20,** 1163

KWAPINSKI, J. B. G., and CHENG, A. (1975) *Ibid.*, **21,** 332

WEBER, K., and OSBORN, M. (1969) *J. biol. Chem.*, **244,** 4406

WEEKE, B. (1973) *Scand. J. Immunol.*, **2,** Suppl. 1, p. 47